## Synopsis of the thesis

**Study on the mechanism of initiator tRNA selection on the ribosomes during translation initiation and rescue of the stalled ribosomes by SsrA in *Escherichia coli.***

The studies reported in this thesis describe the work done in the area of translation initiation where a previously unknown role of multiple copies of initiator tRNA in *E. coli* has been reported. Also the role of SsrA resume codon in resumption of translation, until not clearly known has been reported here. **Chapter -1** discusses the relevant literature in understanding translation and initiator tRNA selection on the ribosome during initiation. It also discusses the literature pertaining to the aspect of release of stalled ribosomal complexes by SsrA. This is followed by the next chapter **(chapter- 2)** which discusses the materials and methods used throughout the study. **Chapter- 3** describes the studies leading to the role of multiple copies of initiator tRNA in *E. coli* in governing the fidelity of initiator tRNA selection on the P site of the ribosome. This is followed by **Chapter-4** which describes the role of the resume codon of the SsrA in governing the efficiency of trans-translation in releasing the stalled ribosomal complexes. The summaries of the **chapters 3** and **chapter 4** are briefly described below.

**i) Role of conserved 3GC base pairs of initiator tRNA in the initiator-elongator tRNA discrimination.**

Translation initiation is the first step in the very important and highly conserved biological process of protein biosynthesis. The process involves many steps, a wide array of protein factors at each specialized step and a large ribonucleoprotein particle; the ribosome to decode the information of the mRNA template into biologically active proteins. The process of initiation is still unclear largely due to fewer reports of available structural data. One of the very

interesting questions that people have been trying to address is how the initiator tRNA is selected on the P- site of the ribosome and what is the importance of the conserved three GC base pairs in the anticodon stem of the initiator tRNA. Here in this study, I have studied this question by using the classical genetic technique of generating and characterizing the mutant initiator tRNA defective at the step of initiation. I have identified and analyzed the suppressors which are capable of rescuing this defect in initiation. The study involves two such *E. coli* suppressor strains (named D4 and D27). These suppressors can initiate translation from a reporter CAT mRNA with amber codon, independent of the presence of the three consecutive GC base pairs in the anticodon stem of initiator tRNAs. Mapping of the mutations revealed that the mutants are defective in expression of the $tRNA_1^{fMet}$ (*metZVW*) gene locus which encodes the initiator tRNA. Both the suppressors (D4 and D27) also allow initiation with elongator tRNA species in *E. coli*. Taken together, the results show that *E. coli* when deficient in the initiator tRNA concentration can lead to initiation with elongator tRNA species.

**ii) The Role of SsrA/tmRNA in ribosome recycling and rescue.**

Occasionally during the process of translation, the ribosomes stall on the mRNA before the polypeptide synthesis is complete. This situation is detrimental to the organism because of the sequestration of the tRNAs as 'peptidyl tRNAs' and the ribosomes. In *E. coli* one of the pathways to rescue stalled ribosomes involves disassembly of these stalled complexes to release peptidyl tRNAs which are then recycled by peptidyl tRNA hydrolase (Pth), an essiential enzyme in *E. coli*. The other pathway which is not essential in *E. coli* but is conserved in all prokaryotes involves SsrA or tmRNA (transfer messenger RNA). The tmRNA is charged with alanine and recognizes the stalled ribosomal complexes and acts as tRNA to bind the A-site. It also functions as mRNA by adding a undecapeptide (which is actually a tag for degradation by cellular

proteases) to the existing polypeptide and there is normal resumption of the translation. In most sequences of SsrA ORF, the first codon of the ORF, called as resume codon, is conserved. I wanted to understand the importance of the conservation of the resume codon. Towards this end I randomly mutated the resume codon and studied the effect of the altered resume codon in the rescue of stalled ribosomal complexes. The effect of over-expression of these mutants was investigated in the rescue of the $Pth^{ts}$ defect since it is known that the overexpression of SsrA rescues the temperature sensitive phenotype of the $Pth^{ts}$ strain and so causes less accumulation of peptidyl–tRNA in *E. coli* .The effect for these mutants has also been studied by the growth of hybrid $\lambda_{imm}P_{22}$ phages. I also used AGA minigene system to study the effect of various mutants which has been shown to sequester $tRNA^{Arg\ (UCU)}$ in the ribosomal P-site, translation of this minigene causes toxicity to *E. coli*. I have tried to study the effect of the SsrA mutants in rescue of toxicity caused by the minigene. Overall, the observations indicate that the conservation of the resume codon is important in *E. coli* and having mutated resume codon probably leads to deficient trans-translation during one or the other growth conditions.